9L.3 Mitochondrial generated nitric oxide protects against permeability transition *via* formation of membrane protein S-nitrosothiols

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Mitochondria generated nitric oxide (NO) regulates several cell functions including energy metabolism, cell cycling, and cell death. Here we report that the NO synthase inhibitors (l-NAME, l-NNA and l-NMMA) administered either *in vitro* or *in vivo* induce Ca^{2+}-dependent mitochondrial permeability transition (MPT) in rat liver mitochondria *via* a mechanism independent on changes in the energy state of the organelle. MPT was determined by the occurrence of cyclosporin A sensitive mitochondrial membrane potential disruption followed by mitochondrial swelling and Ca^{2+} release. In *in vitro* experiments, the effect of NOS inhibitors was dose dependent (1 to 50 μM). In addition to cyclosporin A, l-NAME induced MPT was sensitive to Mg^{2+} plus ATP, EGTA, and to a lower degree, to catalase and dithiothreitol. In contrast to l-NAME, its isomer d-NAME did not induce MPT. l-NAME induced MPT was associated with a significant decrease in both the rate of NO generation and the content of membrane protein S-nitrosothiol. Acute and chronic *in vivo* treatments with l-NAME also promoted MPT and decreased the content of mitochondrial protein S-nitrosothiol. SNAP (a NO donor) prevented l-NAME mediated MPT and reversed the decrease in the rate of NO generation and in the content of membrane protein S-nitrosothiol. We propose that S-nitrosylation of critical membrane protein thiols by NO protects against MPT.

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Posters

9P.1 Stigmatellin as a modulator of metal-induced inner mitochondrial membrane permeabilization

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Previously on two rat cell lines, AS-30D and PC12, we have shown that stigmatellin (an inhibitor of mitochondrial respiratory complex III) is one of the strongest protectors against Cd^{2+}-induced cytotoxicity, in addition to N-acetylcysteine and several mitochondrial permeability transition (MPT) pore inhibitors, namely bongkrekic acid (BKA) and cyclosporine A (CsA). To better understand the molecular mechanisms of the preventive action of stigmatellin, we tested its effectiveness against mitochondrial membrane permeabilization produced by such heavy metals as Cd, Hg, Cu, and Zn, as well as by Ca (in the presence of Pi) or Se (added as Na_2SeO_3), using isolated rat liver mitochondria as a model system. The conducted experiments showed that stigmatellin exhibited the modulating effects on the mitochondrial swelling induced by these metals/metalloids in isotonic sucrose medium in the presence of Asc and TMPD (complex IV substrates) added for energization of the mitochondria in order to bypass the respiratory complexes I, II, and III inhibited by Cd^{2+} etc. In particular, stigmatellin sharply enhanced the mitochondrial swelling, evoked by selenite; however, in the same medium and under the same conditions stigmatellin as well as BKA and CsA did not produce significant effect on Cu^{2+}-induced swelling of isolated rat liver mitochondria in contrast to the high-amplitude swelling produced by Cd^{2+}, Hg^{2+}, Zn^{2+}, or Ca^{2+} plus Pi, which significantly depressed by these inhibitors. In the light of own results and data from literature obtained during the latest time, the hypothesis suggested by us earlier (Belyaeva (2004) Mitochondrion 4: 71; Belyaeva et al., (2004) Chem.-Biol. Interact. 150: 253–270) about the possible involvement of the electron transport chain supercomplex, formed by complex I (P-site) and complex III (S-site) in the mitochondrial membrane permeabilization mediated by the MPT pore is discussed.

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9P.2 MitoTeas: *Vaccinium myrtillus* and *Geranium robertianum* decoctions improve diabetic Goto–Kakizaki rats hepatic mitochondrial oxidative phosphorylation

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Diet-induced metabolic syndrome, leading to obesity, insulin resistance, type 2 diabetes and related diseases are major health problems all over the world, nowadays [1,6]. A common feature to these metabolic alterations is lower mitochondrial oxidative phosphorylation (OXPHOS) enzymatic complexes activities [5]. Several chemical compounds found in plant products had proven to possess beneficial properties, being currently pointed out due to their pharmacological potential in metabolic syndrome complications [2]. In this context, we studied the effect of *Vaccinium myrtillus* and *Geranium robertianum* leaf decoctions on Goto-Kakizaki (GK) rats, a type 2 diabetes mellitus animal model. Our results show that *V. myrtillus* and *G. robertianum* leaf decoctions present significant benefits on glycaemic control and that GK rats treated during four weeks with *V. myrtillus and G. robertianum* decoctions presented an improvement of the evaluated mitochondrial respiratory parameters (state 3, state 4, RCR and FCCP stimulated respiration). These increased OXPHOS activities can be correlated to the high contents of quercetins found in *V. myrtillus* and homoeriodictyol found in *G. robertianum,* that are reported to account for increased protein expression [3,4]. Therefore, these “MitoTeas” seem to be promising therapeutic agents to type 2 diabetes, regarding their high antioxidant activity coupled to their beneficial effects on glycaemic control and mitochondrial activity.

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